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# Negative Gravitropism in Dark-Grown Gametophytes of the Fern *Ceratopteris richardii*

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Abstract.—This study examined whether gravity influences the growth direction of dark-grown gametophytes of the fern *Ceratopteris richardii*. Analyses of directional growth of gametophytes in response to gravitropic stimulation demonstrated that gametophytes showed negative gravitropism. Dark-grown gametophytes of *dkg1 her1* mutants, which germinate in complete darkness, displayed a more distinct negative gravitropism. Unlike *her1* spores, *dkg1 her1* spores do not require light irradiation to induce spore germination. Therefore, light irradiation on *her1* spores was possibly inhibiting the negative gravitropism of *her1* gametophytes. In the present study, prolonged white-light irradiation on *her1* spores inhibited negative gravitropism in the gametophytes. Light irradiation on spores therefore affects the later negative gravitropism of dark-grown gametophytes.

Key Words.—*Ceratopteris richardii*, gametophyte physiology, gravitropism

Spore germination is the first event in the life cycle of ferns. Germinated spores progress autotrophically through many developmental stages to form a mature gametophyte with rhizhoids and gametangia (Momose, 1967; Raghavan, 1989). During this time many environmental factors influence development and morphogenesis of the fern gametophyte. In vascular plants gravity is an important factor controlling plant morphogenesis and directional growth (Morita and Tasaka, 2004; Hoson *et al.*, 2010); similar responses in fern gametophytes have not yet been described in detail.

The fern *Ceratopteris richardii* Brogn. is often used as a plant model system (Hickok *et al.*, 1995; Banks, 1999; Salmi *et al.*, 2005). In the germinating spores of *C. richardii*, Edwards and Roux (1994, 1998) found that the primary rhizoid emerged in a downward direction with respect to gravity, suggesting that germinating spores could sense the direction of gravity. After germination the rhizoid failed to respond to changes in gravity, indicating that the rhizoid itself was not gravitropic (Edwards and Roux, 1994). Gravitropism in *Ceratopteris richardii* gametophytes other than in the rhizoids has not yet been examined. Investigation of gravity sensing by gametophytes is of interest because it is a poorly understood environmental response in gametophyte development. If *C. richardii* gametophytes can sense the direction of gravity and then show gravitropism, the gametophytes will be useful for investigating mechanisms of

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gravitropic responses in non-vascular plants. In the present study, *C. richardii* gametophytes were examined for directional gravitropic responses.

## Materials and Methods

Morphogenesis between male and hermaphroditic gametophytes varies greatly in *Ceratopteris richardii* (Kamachi *et al.*, 2004). Male gametophytes, which are induced by the pheromone antheridiogen (Kamachi *et al.*, 2007), were insensitive to light for induction of asymmetric cell division followed by rhizoid development (Murata and Sugai, 2000), suggesting that male gametophytes might be less sensitive to environmental changes. Therefore *her1* mutants, which are antheridiogen-insensitive mutants and do not develop into males (Banks, 1994), were used for this work. In this study, *dkg1 her1* double mutants were also used. The *dkg1* (*dark-germinator 1*) mutant allele enables the spore to germinate in complete darkness (Scott and Hickok, 1991; Kamachi *et al.*, 2004).

*Ceratopteris richardii* spores of *her1* and *dkg1 her1* mutants were collected from fertile fronds in a greenhouse at Toyama University. The spores were sterilized for 3 min in commercial 5% NaOCl bleach, 0.02% (*w/v*) Triton X-100, rinsed with distilled water, and incubated in the dark for 7 days to synchronize germination. Spores were then irradiated for 24 h at 26°C under white light (5.0 J $m^{-2}$ $s^{-1}$), and germinated in the dark to obtain strap-shaped gametophytes (Fig. 1B). Spores of the *dkg1 her1* mutants were germinated in the dark immediately after the sterilization because these mutants can germinate in the dark. A 1:4 dilution of Murashige and Skoog (MS) salt mixture (Wako Pure Chemical Industries, Osaka, Japan) solidified with 0.3% (*w/v*) Bacto Agar (Difco) was used as the germination medium.

Gravitropism of *Ceratopteris richardii* gametophytes was evaluated in 9-day old gametophytes grown on agar medium in the dark. Observations were made using a stereoscopic zoom microscope (Nikon, SMZ-1000). In each experiment 50–100 gametophytes were classified into three types: gametophytes that grew

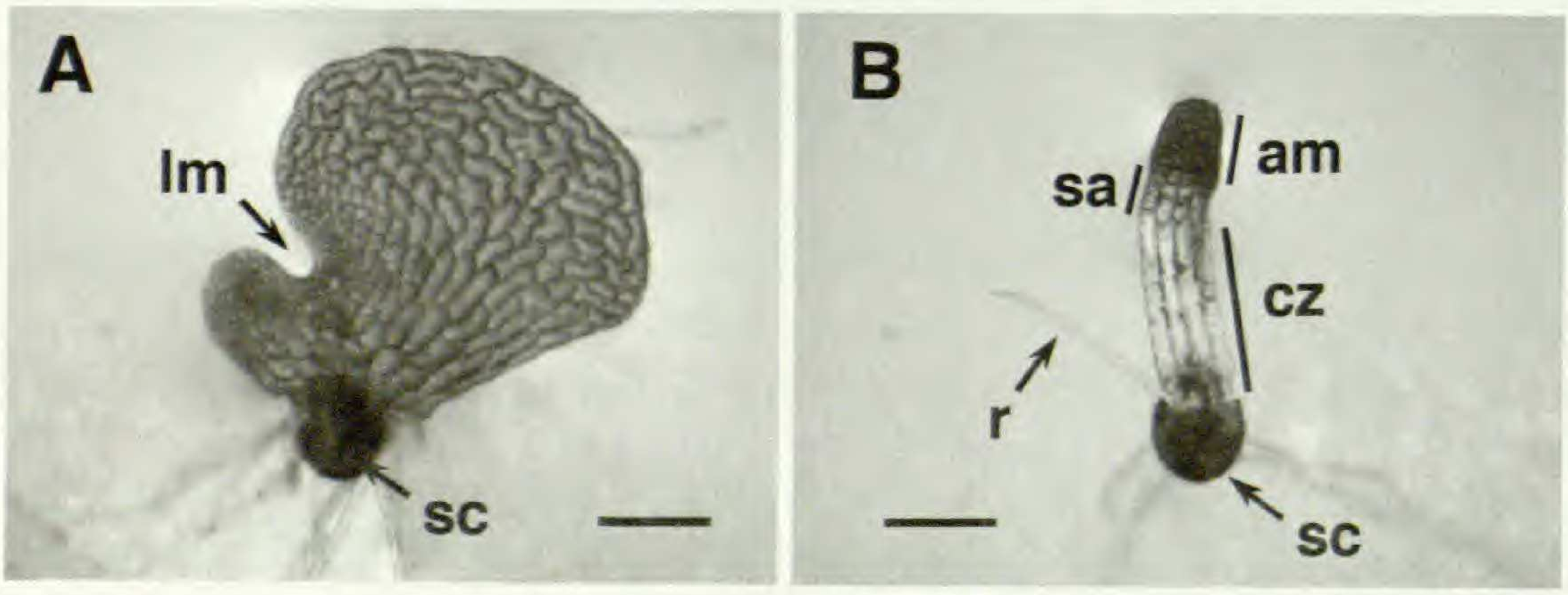


Fig. 1. Morphological profiles of 7-d-old *Ceratopteris richardii* gametophytes grown in the light (A) and in the dark (B). *am* Apical meristem, *cz* basal growth cessation zone, *lm* lateral meristem, *r* rhizoid, *sa* subapical elongation zone, *sc* spore coat. Scale Bar = 0.2 mm.

upward, downward, and horizontally with respect to the surface of the agar medium. Results were expressed as mean values of percentages obtained from three separate experiments.

## RESULTS

Figure 1 shows typical morphological profiles of 7-day old *Ceratopteris richardii* gametophytes grown in the light (A) and dark (B). The dark-grown gametophytes have a strap-shape with 3–6 rows of cells in a single plane, an apical meristem, a subapical elongation zone, and a basal growth cessation zone where the cells are extremely elongated. A similar growth habit was also described in a study by Murata *et al.* (1997).

Dark-grown gametophytes were first examined for a display of gravitropism. Sixty one percent of the 8-day old, dark-grown gametophytes grew upward with respect to gravity, and 10% grew downward (Fig. 2), which suggests that *Ceratopteris richardii* gametophytes display a tendency toward negative gravitropism. For a clearer demonstration of negative gravitropism, gametophytes were turned upside down one and two days before observation. These gametophytes changed their direction of growth from "upward" to "downward" following this rotation (Fig. 2), which further demonstrates that *C. richardii* gametophytes display negative gravitropism.

Figure 2 shows results from gametophytes with the *dark germinator 1* (*dkg1*) mutant allele, which enables spores to germinate in complete darkness (Scott and Hickok, 1991; Kamachi *et al.*, 2004). Interestingly, 89% of these gametophytes grew upward, and only 1% grew downward. Thus, these mutants showed an enhancement of negative gravitropism compared with the

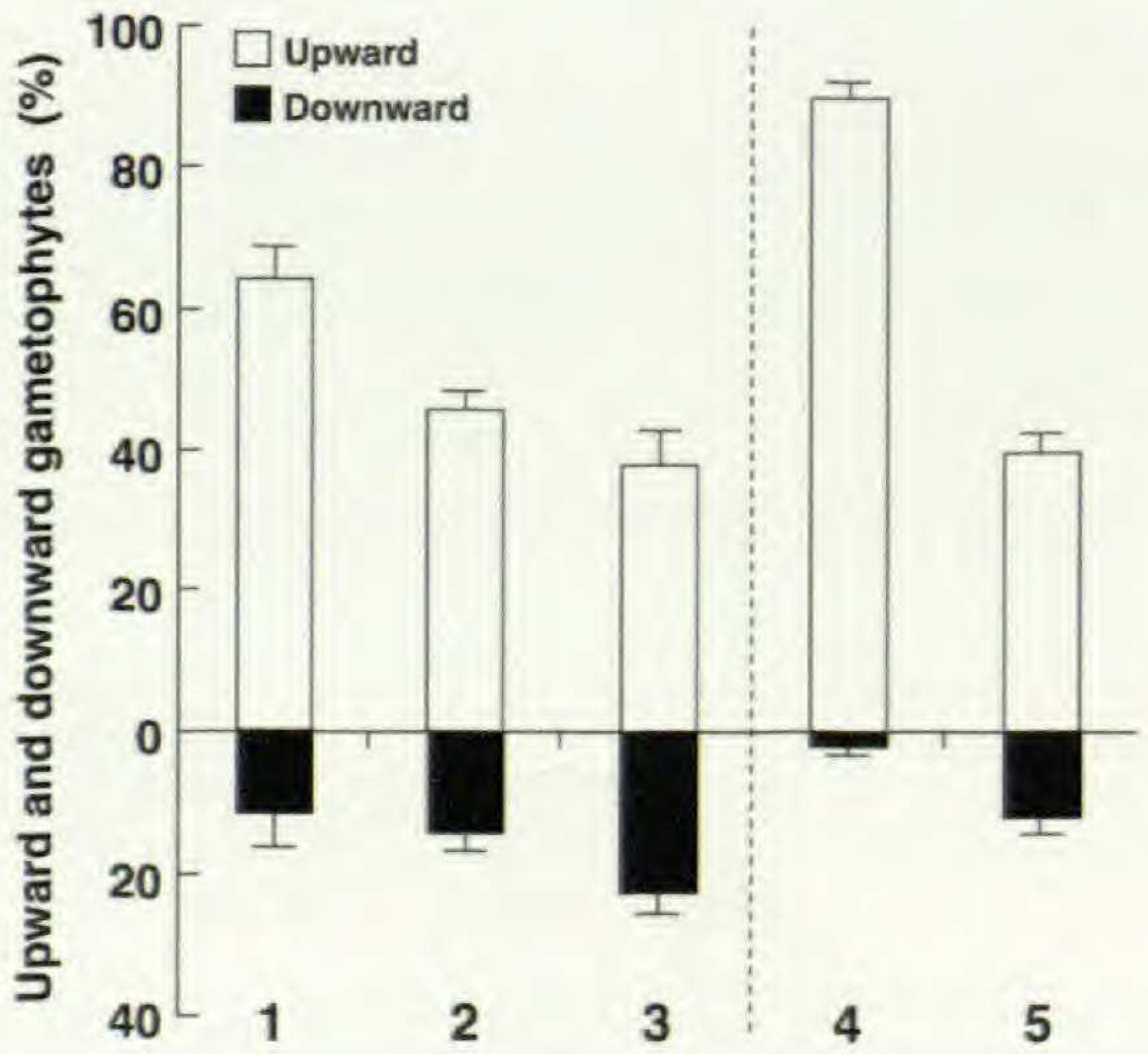


FIG. 2. Percentages of dark-grown *Ceratopteris richardii* gametophytes that grew upward and downward directions in *her1* mutants (1, 2, 3) and *dkg1 her1* mutants (4, 5). Gametophytes grown on agar medium placed horizontally (1 and 4); gametophytes turned upside down one day before the observation (2); gametophytes turned upside down two days before the observation (3 and 5). Values represent the means evaluated from three separate experiments. In each experiment 50–100 gametophytes were observed. Bars are standard errors.

*her1* mutants. These results imply that white light, which is required to induce spore germination, may inhibit the subsequent gravitropism of developing gametophytes.

To confirm this hypothesis, white-light irradiation effects on gravitropism were examined using *her1* gametophytes (Fig. 3). When the length of irradiation time was changed from 18 h to 48 h, the negative gravitropic response weakened. Seventy-four percent of gametophytes grew upward when the irradiation time was 18 h, as opposed to 48% when irradiation time was extended to 48 h. On the other hand, the percentages of gametophytes that grew downward and horizontally increased to 14 and 35% from 4 and 22%, respectively, when the length of irradiation time was changed from 18 h to 48 h. Thus, the white light irradiation during the initial developmental steps in spore germination inhibited negative gravitropism in *Ceratopteris richardii* gametophytes.

## Discussion

This research was conducted to determine whether gravity affects the directional growth of dark-grown gametophytes of *Ceratopteris richardii*. Gametophytes showed negative gravitropism similar to that as seen in most seedlings of vascular plants, caulonema of the moss *Physcomitrella patens*

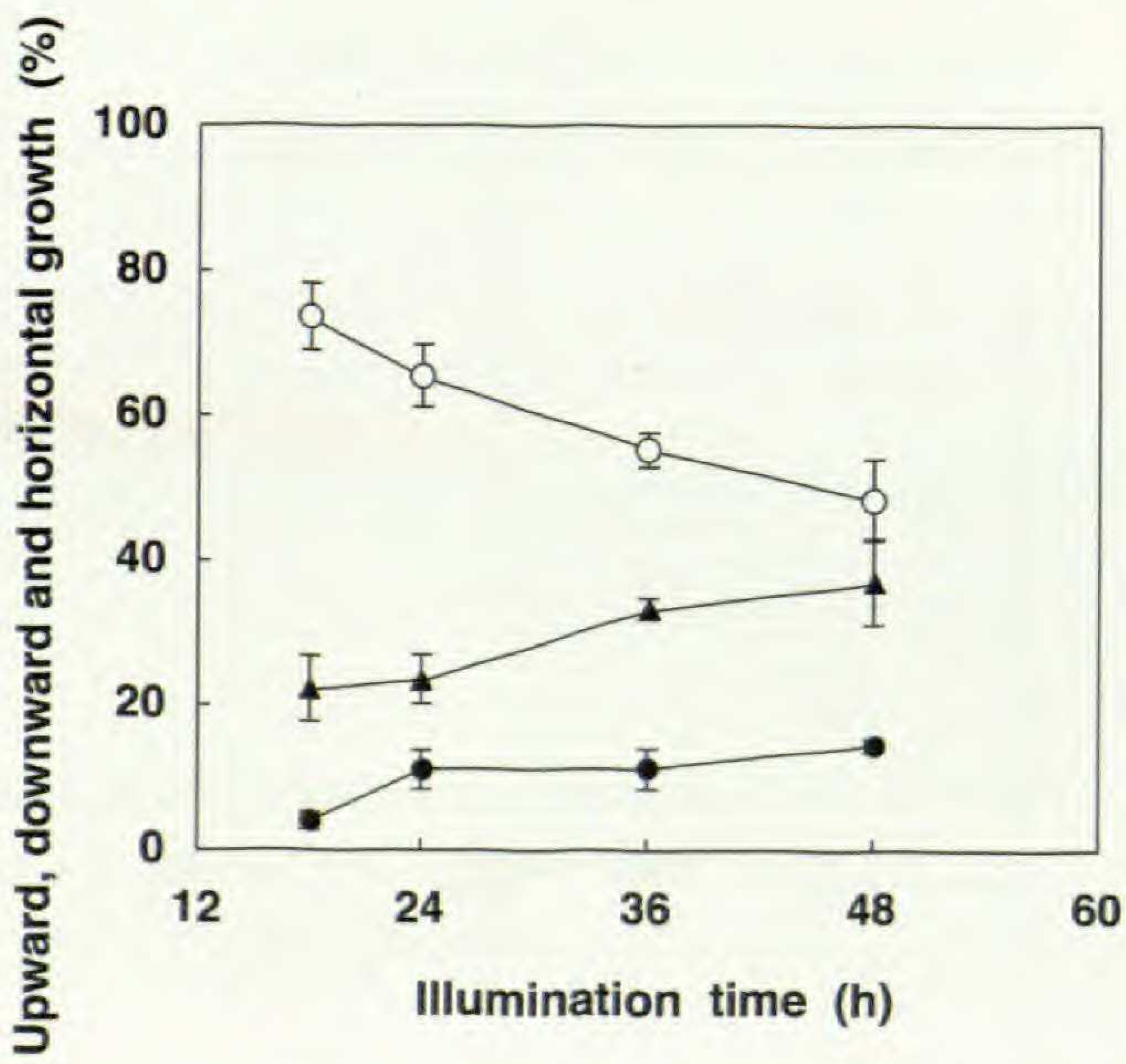


Fig. 3. White-light irradiation effects on negative gravitropism in *Ceratopteris richardii* gametophytes. The *her1* spores were irradiated by white light for the designated times to induce spore germination, then germinated and grown in the dark. Percentages of gametophytes that grew upward (open circles), downward (closed circles) and horizontally (closed triangle) were determined from the 9-d-old gametophytes. Values represent the means evaluated from five separate experiments. In each experiment 50–100 gametophytes were observed. Bars are standard errors.

(Martin *et al.*, 2009), and protonemata of characean algae (Braun and Limbach, 2006).

Gravitropism in plants occurs in three temporal stages: gravity perception, signal transduction, and organ response (Kumar *et al.*, 2008). The detailed mechanisms of gravity perception have been unveiled mostly in vascular plants. In *Arabidopsis thaliana* (L.) Heynh. amyloplast movement along the gravity vector within gravity-sensing cells is the most likely trigger of a subsequent gravitropic response (reviewed in Morita and Tasaka, 2004). In contrast, no data are available to explain how *Ceratopteris richardii* gametophytes might sense the direction of gravity. Amyloplasts would not seem to be involved in gravity perception in *C. richardii* gametophytes because no starch-accumulating amyloplasts were found in dark-grown gametophyte cells following $I_2$-KI staining (data not shown). This suggests the involvement of some other statolith in triggering the gravitropic response in *C. richardii* gametophytes.

Edwards and Roux (1994) found that germinating spores of *Ceratopteris richardii* could sense the direction of gravity because gravity directed the nuclear migration in the germinating spores, as well as the initial direction of growth of the primary rhizoid. They detected a calcium flux in the germinating spores as the earliest gravity-directed event (Chatterjee *et al.* 2000), suggesting that calcium channels and pumps may be involved in the primary gravity perception mechanism in *C. richardii* spores. Recently, Salmi *et al.* (2011) proposed that the gravity-directed calcium current is regulated primarily by the activation of mechanosensitive calcium channels at the bottom of the spore, based on data obtained from a silicon microfabricated sensor array. Thus, the nuclear migration and the following calcium flux might be important in the gravity perception mechanism in *C. richardii* gametophytes.

As shown in Figure 3, the white-light irradiation that is required to induce spore germination weakened the negative gravitropism in *her1* gametophytes, indicating that light irradiation on spores influences the later negative gravitropism of dark-grown gametophytes. In fact, the dark-grown gametophytes with the *dkg1* mutant allele showed distinct negative gravitropism as compared with the gametophytes without the *dkg1* allele (Fig. 2). The *dkg1* mutants were shown to be constitutively active in several photomorphogenic responses mediated by phytochrome (a red and far-red light photoreceptor) through the gametophytic phase (Kamachi *et al.*, 2004), in addition to the dark-germinating property. Considering these characteristics of the *dkg1* mutants, phytochrome may not be responsible for the inhibitory effect of white light on subsequent negative gravitropism. In preliminary experiments, blue- and green-light, but not red light, affected the inhibition of negative gravitropism (Adachi and Kamachi, unpublished data).

The gravitropic growth-orientation of the seedlings of flowering plants is also inhibited by light (Correll and Kiss, 2002). In contrast to *C. richardii* gametophytes, however, phytochrome is responsible for the inhibition of gravitropism in *Arabidopsis thaliana* (Poppe *et al.* 1996; Lariguet and Fankhauser, 2004), where phytochrome is found to promote the conversion of amyloplasts to other forms of plastids in the endodermis, causing cessation of hypocotyl gravitropism (Kim *et al.*, 2011). Thus, the mechanisms involved in the light-induced inhibition of negative gravitropism in *C. richardii* gametophytes are likely to be different than those operating in *A. thaliana* seedlings. Further analyses are required to determine how the negative gravitropism of the gametophytes is inhibited by light and to identify the gravity-sensing mechanisms of *C. richardii* gametophytes.

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